

Short communication

Effects of seed storage and fire on germination in the nut-fruited
Restionaceae species, *Cannomois virgata*R.J. Newton^a, W.J. Bond^a, J.M. Farrant^{b,*}^a Department of Botany, University of Cape Town, Private Bag, Rondebosch 7701, South Africa^b Department of Molecular and Cell Biology, University of Cape Town, Private Bag, Rondebosch 7701, South Africa

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Abstract

The effects of storage regime (laboratory and soil storage) and fire cues (heat and charate) on seed viability and germination were investigated in the nut-fruited Restionaceae species, *Cannomois virgata* (Rottb.) Steud. Soil-stored seeds were cycled through a series of alternating temperature and moisture regimes in a phytotron, while laboratory-stored seeds were kept at comparatively constant temperatures and low humidity. Seed deterioration in soil-stored seed was not significantly different to laboratory-stored seed. A marked improvement in germination of soil-stored seed was observed on exposure to charate from a fire.

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Germination of seeds of the nut-fruited Restionaceae has been notoriously difficult to achieve (Brown et al., 1994; Brown et al., 1998). Poor seed germination may be due to inappropriate seed collection methods (because of lack of information on seed maturation and release) or sorting methods, resulting in poor seed quality (Clegg, 1980; Brown et al., 1998). However, recent research into the suitability of seed collection and sorting methods, seed quality and viability suggest that seed germination remains poor even in well-sorted seed collections (Newton et al., 2002). It is therefore likely that poor seed germination in these collections is due to seed dormancy mechanisms.

Cannomois virgata (Rottb.) Steud. occurs in the species-rich fynbos of the southwestern Cape. Environmental factors that characterize the fynbos are the Mediterranean winter rainfall and summer drought, nutrient poor soils and periodic disturbance by fire (Taylor, 1978; Bond and Goldblatt, 1984; Kruger and Bigalke, 1984). Many of the nut-fruited Restionaceae possess a fleshy oil body (elaiosome) that is attractive to ants (Bond and Slingsby, 1983). Ants collect these seeds from the soil surface and bury them underground at depths of 40–70 mm (Bond et al., 1991) where they are protected from detrimental

surface disturbances such as fire (de Lange and Boucher, 1993) and rodent predation (Bond and Breytenbach, 1985).

Fire plays an important role in the fynbos, directly influencing plant growth, survival and reproduction. Fire also impacts upon seed and seedling dynamics (Bond and van Wilgen, 1996). Plant survival strategies following fire may be vegetative (e.g. resprouting) and/or reproductive (e.g. fire-stimulated seed germination) (Bond and van Wilgen, 1996; van Staden et al., 2000). In the nut-fruited Restionaceae, both resprouting and seed germination following fire have been observed (Linder, 1991).

Fire-stimulated seed germination is important in Mediterranean-type ecosystems around the world. Species in which seed germination is directly or indirectly stimulated by the occurrence of fire typically possess soil-stored seeds that require a fire cue for germination. These seeds are thought to be long-lived, and consequently maintain a persistent seed bank (Keeley, 1994). Fire-linked germination cues have been studied extensively in flora of the Californian chaparral (Keeley, 1991; Thanos and Rundel, 1995), Australian shrublands (Bell et al., 1993; Dixon et al., 1995; Roche et al., 1997), Mediterranean shrublands (Parker and Kelly, 1989; Roy and Sonié, 1992) and fynbos (Brown and van Staden, 1997; Keeley and Bond, 1997; Brown et al., 2003).

Many laboratory germination tests by various researchers have been conducted on *C. virgata* and other nut-fruited

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Restionaceae species with little or no germination success (reviewed in Newton, 2000). Although a significant improvement in germination (from 2% to 18%) was noted in *C. virgata* by Brown et al. (1994) when seeds were subjected to smoke treatment, 82% did not germinate. It is unlikely that this lack of germination was due to poor seed collection techniques as the authors were aware of the difficulties surrounding seed collection. Smoke has been suggested to be one of a number of germination cues of importance in this species (Brown and Botha, 2004).

After-ripening is a process in which dry dormant seeds slowly lose dormancy over time (Bewley and Black, 1994). Wet–dry cycles are also required for the loss of dormancy in some species (Baskin and Baskin, 1998). As *C. virgata* seeds are buried by ants they would be exposed to wet–dry cycles and temperature fluctuations in the soil. The effects of after-ripening and soil storage on *C. virgata* seeds have not been investigated.

The aim of this research was to investigate the effects of soil storage and dry after-ripening (laboratory storage) coupled with cues of heat and charate on seed germinability of the nut-fruited *C. virgata*.

C. virgata seed was collected from three populations in the southwestern Cape. Seed collection and sorting methods are detailed in Newton et al. (2002). Seed quality was checked using X-ray and tetrazolium tests prior to germination experiments (International Seed Testing Association, 1999; Newton et al., 2002).

Laboratory-stored seed was kept in brown paper bags under ambient (average temperature ca. 23 °C) dry laboratory conditions while soil-stored seed was cycled through a series of alternating temperature and moisture regimes described below.

Soil-stored seed was buried in seed trays containing soil collected from Silvermine Nature Reserve (34°05S, 18°25'E). Silvermine soil is typical of fynbos communities: sandy, acidic and low in nutrients (Newton, 2000). Seeds were buried at least 10 mm below the soil. Soil-stored seed was subjected to a series of alternating temperature and varied watering regimes in a phytotron for a period of 16 weeks (Table 1), simulating fluctuating temperature and rainfall as may be experienced by seeds in the natural environment. At the end of 16 weeks seeds were removed from the soil and checked for germination (identified by radicle protrusion) and deterioration (identified by visual inspection and floating in water following Newton et al., 2002). Germinated and deteriorated seeds were recorded and

discarded and the remaining seeds replaced in the soil. Seeds were cycled twice more through 16 weeks of soil storage as described above. Tetrazolium tests were conducted at the end of 48 weeks of soil storage on a subset of seeds to check viability.

The role of fire as a dormancy-breaking mechanism in soil- and laboratory-stored seeds was examined by simulating the fire conditions that may be experienced by seeds in the natural environment. Three replicates of 300 laboratory-stored seeds and four replicates of 300 soil-stored seeds were used in this experiment. Each batch of 300 seeds was divided into three equal portions of 100 seeds: a control, charate-treated and fire-treated seed.

Fire-treated seeds were exposed to charate and heat from a fire. Seeds were placed in small bags of plumber's mesh (gauze bags which allowed seeds to be in contact with the soil) and buried 40 mm below the soil surface in a metal container (1 m × 1 m × 0.15 m) containing Silvermine soil. Approximately 0.14 m³ of dead brush and fresh fynbos vegetation consisting primarily of *Passerina vulgaris* Thoday (Thymelaceae) and *Restio triticeus* Rottb. (Restionaceae) was collected as fuel. The brush was spread out evenly over the soil surface. Following fire ignition, surface and soil temperatures were monitored until temperatures returned to ambient levels. Probes of a K-type Thermocouple (Fluke, Massachusetts, USA) were buried in the soil at the same depth as the seeds to measure soil temperatures. A Raynger 3I series Raytec Infrared Thermometer (EPD Technology Corp., New York, USA), set to an emissivity of 0.95, was used to measure the surface temperature of the fire.

Charate-treated seeds were exposed to charate but not heat. These seeds were buried in soil that had been exposed to the fire. Surface charate was included in these seed trays. Control seeds, which were exposed to neither charate nor heat, were buried in seed trays in untreated Silvermine soil.

Seed trays containing fire-treated, charate-treated and control seeds were placed in a phytotron for 1 week without watering at 10 °C, 12 h/30 °C, 12 h to simulate high autumnal temperature fluctuations that would be experienced by seeds following vegetation removal by fire. The phytotron was then set to winter germination conditions (10 °C, 16 h/20 °C, 8 h) and seeds watered twice a week for a period of 10 weeks. Technical problems in the phytotron resulted in a temperature spike at the end of the second week. However this did not appear to affect germination.

At the end of the experiment, germinants and visibly deteriorated seed were recorded, and a subset of the remaining seed was tested for viability with tetrazolium. A two-factor analysis of variance was used to test for significant differences in arc-sine transformed germination and deterioration. Results from the three populations of *C. virgata* were combined prior to statistical analysis. Significantly different values were separated using Scheffé's multiple range tests (Zar, 1996).

Germination during soil storage was less than 2% (Newton, 2000). The percentage of non-viable seeds at the end of soil storage (determined from visually examining and floating all seeds removed from the soil) was 3.8 ± 1.5%. Tetrazolium testing showed that all whole seeds were viable ($n=20$).

Table 1
Alternating temperature and water regimes for soil-stored seeds in the phytotron

Duration	Alternating temperatures	Watering
4 weeks	20 °C, 10 h/30 °C, 14 h	Single wetting at the beginning of week 3
2 weeks	5 °C, 12 h/15 °C, 12 h	Twice a week
8 weeks	10 °C, 16 h/20 °C, 8 h	Twice a week
2 weeks	20 °C, 14 h/30 °C, 10 h	Once a week

Freely draining seed trays were watered to saturation with a fine-spray hose with frequencies indicated in the table.

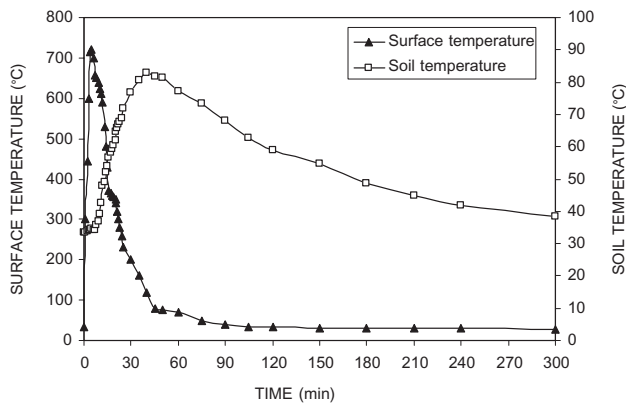


Fig. 1. Surface and soil temperatures (40 mm below the surface) during the fire experiment.

Surface and soil temperatures 40 mm below the surface during the fire are shown in Fig. 1. The maximum average surface temperature was in excess of 700 °C. However, these high surface temperatures only persisted for about 10 min. The soil temperatures at the level at which the seeds were buried rose to a maximum average temperature of 83 °C, and returned to the original levels 4 h after fire ignition. These temperatures are representative of an intense hot fynbos burn (Kruger and Bigalke, 1984; de Lange and Boucher, 1993).

Seed germination was significantly different in both storage regimes and dormancy-breaking treatments (Fig. 2). Lowest germination occurred in laboratory-stored seed. Germination of soil-stored seed was significantly higher than laboratory-stored seed within the control, charate and fire treatments. This suggests that there may be a gradual release from dormancy in soil-stored seeds over time. Charate and fire treated seeds also exhibited significantly higher germination than the control. Highest germination was observed in seeds that were exposed to charate after a period of storage in the soil. These germination percentages are substantially higher than smoke-treated *C. virgata* in the previous study by Brown et al. (1994), while germination in control laboratory-stored seeds was similar.

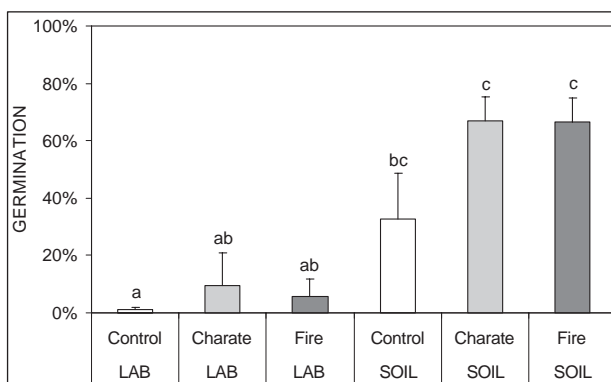


Fig. 2. Mean percentage germination of control, charate-treated and fire-treated (charate+heat) seeds of laboratory- and soil-stored *C. virgata* following the fire experiment ($n \geq 3$ replicates of 100 seeds). A two-factor analysis of variance on arcsine transformed germination percentages showed that storage conditions (laboratory or soil) and treatment (control, charate or fire) were both significantly different (storage $F=103.21$, treatment $F=6.51$, $P<0.01$). There was no significant interaction between storage and treatment ($F=1.25$, $P=0.32$).

Conditions during soil storage seem to prepare the seeds for germination. This may constitute a release from dormancy related to changes in temperature and/or rainfall, changes in the soil or a combination of the above. Following a fire, at the onset of winter rains, charate (and smoke) components will penetrate the soil and are thought to break the remaining physiological dormancy in *C. virgata*, resulting in mass seed germination. Similar requirements for germination consisting of a period of soil storage followed by a brief charate or smoke treatment have been reported in species indigenous to other Mediterranean climate regions, such as the post-fire germinating chaparral species *Dicentra chrysantha* (Keeley and Fotheringham, 1998) and several southwestern Australian species (Tieu et al., 2001). However, germination in fire-treated seed was not significantly different from germination in charate-treated seed, indicating that the heat shock did not affect germination.

Seed deterioration was significantly different in dormancy-breaking treatments but not storage regimes (Fig. 3). Whether seeds were stored in the laboratory or the soil, they exhibited similar deterioration percentages. However, deterioration of charate-treated and fire-treated seed was significantly higher than control seed. It is likely that some of the seeds scored as ‘deteriorated’ commenced germination, but were unable to complete this process to become a seedling. On removal of the seeds from the soil at the end of 10 weeks at winter germination conditions, it was impossible to determine the cause of seed deterioration. It is unlikely that heat from the fire contributed to seed deterioration as fire-treated seeds did not deteriorate significantly more than charate-treated seeds. The finding that fire temperatures did not damage seed is consistent with other studies that have reported seed survival at temperatures higher than recorded in this study (e.g. Musil and de Witt, 1991; de Lange and Boucher, 1993).

It appears that the soil seed bank of *C. virgata* is built up by ant-dispersal during non-fire years. Repeated wetting and drying and temperature fluctuation during soil storage did not decrease seed viability, suggesting that repair processes are

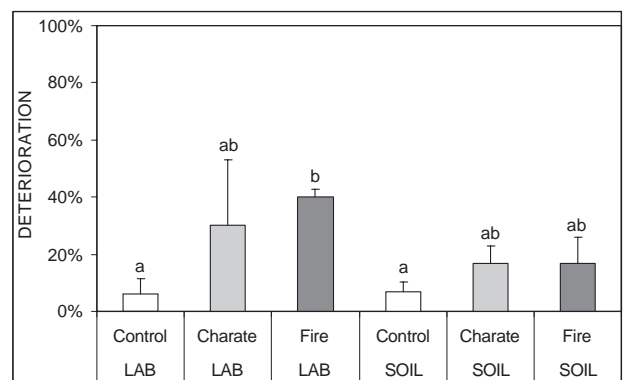


Fig. 3. Mean percentage deterioration of control, charate-treated and fire-treated (charate+heat) seeds of laboratory- and soil-stored *C. virgata* following the fire experiment ($n \geq 3$ replicates of 100 seeds). A two-factor analysis of variance on arcsine transformed deterioration percentages showed that treatment (control, charate or fire) was significantly different ($F=11.48$, $P<0.01$) but that storage conditions (laboratory or soil) was not significantly different ($F=5.64$, $P=0.03$). There was no significant interaction between storage and treatment ($F=2.39$, $P=0.13$).

operating in imbibed soil-stored seeds (Priestley, 1986). Seeds of other fynbos species in the Proteaceae, Geraniaceae and Fabaceae have been found to maintain viability and form persistent seed banks in the soil (Brits, 1996; Holmes and Newton, 2004).

Significant improvement in germination in *C. virgata* was achieved by a charate treatment following a period of soil storage of seeds in alternating temperatures and varied moisture regimes. Future research should include investigation into physiological changes in seeds during soil storage and charate or smoke treatment, which may elucidate mechanisms of seed dormancy release. Studies on seed dormancy mechanisms of other nut-fruited Restionaceae species would complement this study and may lead to the emergence of patterns in germination strategy in species sharing a similar ecology.

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